Immigration delays but does not prevent adaptation following environmental change: experimental evidence

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Running head: **Evolutionary rescue with immigration**

Abstract

In today's rapidly changing world, it is critical to examine how animal populations will respond to severe environmental change. Following events such as pollution or deforestation that cause populations to decline, extinction will occur unless populations can adapt in response to natural selection, a process called evolutionary rescue. Theory predicts that immigration can delay extinction and provide novel genetic material that can prevent inbreeding depression and facilitate adaptation. However, when potential source populations have not experienced the new environment before (*i.e.,* are naive), immigration can counteract selection and constrain adaptation. This study evaluated the effects of immigration of naive individuals on evolutionary rescue using the red flour beetle, *Tribolium castaneum,* as a model system. Small populations were exposed to a challenging environment, and three immigration rates (zero, one, or five migrants per generation) were implemented with migrants from a benign environment. Following an initial decline in population size across all treatments, populations receiving no immigration gained a higher growth rate one generation earlier than those with immigration, illustrating the constraining effects of immigration on adaptation. After seven generations, a reciprocal transplant experiment found evidence for adaptation regardless of immigration rate. Thus, while the immigration of naive individuals briefly delayed adaptation, it did not increase extinction risk or prevent adaptation following environmental change.

Keywords: evolutionary rescue, dispersal, *Tribolium castaneum*, gene flow, translocation

1 | Introduction

A critical goal of conservation biology is to establish how to effectively manage populations in human-altered habitats. Environmental change such as pollution (Loria *et al.*, 2019), deforestation (Foster *et al.*, 2021), and fragmentation (Cheptou *et al.*, 2017) can reduce habitat quality and connectivity, which can decrease the average fitness of residing populations. If fitness drops below the replacement rate, populations will decline in size and will approach extinction if the downward trend persists. Immigration, either through maintenance of dispersal corridors or translocations, is one approach readily available to conservation practitioners to support declining populations, particularly in fragmented landscapes (Frankham *et al.*, 2019).

Theoretical work within the conservation literature recommends an immigration rate of one to ten migrants per generation for isolated populations (Mills & Allendorf, 1996; Frankham *et al.*, 2019). More specifically, theory suggests a rate of one migrant per generation to offset population divergence and loss of heterozygosity that occurs through drift (Mills & Allendorf, 1996), and five migrants per generation to prevent damaging inbreeding depression and maintain adaptive genetic variation in changing environments (Frankham *et al.*, 2019). Ideally, immigration can occur consistently through dispersal corridors, and if translocations are needed, consistent immigration should be sustained. However, these recommendations have been tested only partially. Laboratory experiments have demonstrated the benefits of immigration when comparing isolated populations to populations that received a single immigration event under scenarios of inbreeding (Spielman & Frankham, 1992) or low diversity populations facing environmental change (Hufbauer *et al.*, 2015). Only one study we know of,

Newman & Tallmon (2001), tested immigration that occurred every generation. They assessed rates of one or 2.5 migrants per generation in recently isolated mustard plant populations, and found that the rate of one migrant per generation was sufficient to maintain genetic variation and decrease inbreeding when compared to isolated populations (Newman & Tallmon, 2001). In this era of global change, it is important to also test these recommendations under more severe environmental conditions.

A separate but related body of literature focuses on how populations declining due to exposure to challenging environments can avoid extinction through adaptive evolution, a process called evolutionary rescue (Gomulkiewicz & Holt, 1995; Carlson *et al.*, 2014). Examples include adaptation in weed or insect populations to pesticides, antibiotic resistance in bacteria, or invertebrate adaptation to metal pollution in streams (Bell, 2017). Experiments show that immigration or admixture can enhance the probability of evolutionary rescue if populations are exposed to a challenging environment (Hufbauer *et al.*, 2015; Durkee *et al.*, 2023). Furthermore, theoretical studies show that the probability of rescue can increase with immigration, primarily due to the added genetic variation, which increases the chance of beneficial alleles being present (Tomasini & Peischl, 2020; Czuppon *et al.*, 2021). To contrast, other theoretical studies show that gene flow from maladapted immigrants can limit adaptation by swamping adaptive alleles in the recipient population, reducing fitness (*i.e.,* outbreeding depression) (Slatkin, 1987; Lenormand, 2002; Brady *et al.*, 2019).

To reduce the likelihood of outbreeding depression, using a source population from a habitat similar to the recipient population (*i.e.,* habitat matching) is recommended by models (Edelaar & Bolnick, 2012) and in the context of conservation (Frankham *et al.*, 2019). However,

88 this is often not possible, particularly under scenarios of novel environmental change (Aitken &
89 Whitlock, 2013) or when the species of concern is rare (Frankham *et al.*, 2019). Even when
90 migrants are not adapted to their new habitat, for example when individuals from a
91 geographically distant population (*e.g.,* Hedrick & Fredrickson, 2010) or from captivity (*e.g.,*
92 Crone *et al.,* 2007) are used, every-generation immigration of one to five individuals is meant to
93 be low enough to minimize outbreeding depression while still maintaining genetic diversity and
94 adaptive potential of populations (Newman & Tallmon, 2001; Frankham *et al.*, 2019). However,
95 to our knowledge, no study has compared the recommended immigration rates of one and five
96 migrants per generation and their impacts on adaptation in the context of severe
97 environmental change.

98 Here, we evaluated how immigration rates recommended in the conservation literature
99 (Frankham *et al.*, 2019) impacted evolutionary rescue (Carlson *et al.*, 2014). To do this, we first
100 created experimental populations of red flour beetles (*Tribolium castaneum*) from a genetically
101 admixed stock population (Durkee *et al.,* 2023). We then introduced them to a challenging
102 environment to mimic a situation where a large, diverse population experiences sudden habitat
103 change that decreases population size and reduces habitat quality, for example when forest are
104 logged (Hillers *et al.,* 2008). Each experimental population received one of three immigration
105 treatments (zero, one, or five individuals every generation), and we observed the effects of
106 immigration on population persistence and growth. In habitats like these that have limited
107 space and degraded resources, population recovery may be constrained by density-dependent
108 processes like competition for food and space (Osmond & de Mazancourt, 2013; Nordstrom *et*
109 *al.*, 2023). Thus, we evaluated both density-dependent and density-independent population

growth through time to tease apart the impacts of negative density dependence and adaptation. Our experiment was designed to address the following question: In populations experiencing severe environmental change, how do different immigration rates affect *(i)* population size and growth rates over time, *(ii)* the timing of evolutionary rescue, and *(iii)* adaptation to the challenging environment?

2 | Methods

To evaluate the question posed in the introduction, we carried out an experiment where we subjected replicated, independent populations to a challenging environment that caused them to decline in size. This environment, therefore, would have led to extinction unless the populations were able to adapt sufficiently to the challenging conditions. Hereafter, we refer to this as the rescue experiment (Figure 1a-b).

Rescue experiment initiation and propagation

We created a genetically diverse *Tribolium castaneum* stock population comprised of lineages described in Durkee *et al.* (2023). The stock population had been reared on standard media (19:1 wheat flour to brewer's yeast) and in standard environmental conditions (31°C and 50-80% humidity) in 4x4x6cm patches for nine, 35-day non-overlapping generations prior to the start of the rescue experiment. To start, we created 25 independent populations from the stock population of 300 individuals each (Figure 1a). These populations were used to initiate replicated experimental populations and maintain populations to serve as a source of migrants

132 (hereafter, source populations). These populations were divided at the start of the rescue
133 experiment, and therefore represent newly isolated populations with no history of divergence,
134 similar to Newman & Tallmon (2001).

135 **Experimental populations** were each initiated with 50 adult beetles, 36 days old, and
136 population sizes were allowed to fluctuate naturally in subsequent generations. Each
137 population was placed onto a single patch of challenging environment created using media
138 containing the pyrethroid insecticide deltamethrin (DeltaDust, Bayer). This event represents a
139 sudden environmental change that reduced fitness and created replicated, isolated
140 populations. The experimental patches each contained a concentration of deltamethrin that a
141 dose-response curve created from preliminary experiments (Figure S1) indicated would
142 decrease the population growth rate to approximately 0.40 from a typical value of around 2.
143 Without adaptation, this reduced growth rate should have led to extinction in 10 generations
144 (based on simulations with the stochastic Ricker model developed for *T. castaneum* by
145 Melbourne & Hastings, 2008). The experimental populations (N=50) were initiated in 25 groups
146 of three, each group from one of the original 25 independent populations (Figure 1a).

147 **Source populations** were initiated with 150 adults, 36 days old, and each served as a
148 source of migrants for a group of three experimental populations (Figure 1a). Thus, 25 source
149 populations were initiated in total, each from one of the original 25 populations. We intended
150 for the sources to represent larger populations, and so we used the large population size
151 (N=150) from Hufbauer *et al.* (2015). Sources were maintained at 150 adults every generation
152 regardless of the number of offspring produced, to approximate populations with a carrying
153 capacity of 150 individuals. Source populations were each maintained on a single patch

154 containing standard dry media, a benign environment for *T. castaneum*. Thus, these individuals
155 were not exposed to the challenging environment present in the experimental patches.
156 Hereafter, we refer to these individuals as naive migrants, to refer to both evolutionary and
157 behavioral naivety.

158 The adult beetles in the experimental and source patches were given 24 hours to mate
159 and oviposit. Adults were then were discarded, and the eggs developed for an additional 34
160 days. The first census event (N_1, Figure 1b) occurred on day 35. Subsequently, one of three
161 immigration treatments was applied to each experimental population in every group: zero, one,
162 or five migrants. The migrants were moved from the source to each experimental patch
163 manually using a paintbrush. This occurred at the start of the following oviposition period. This
164 procedure (immigration of zero, one, or five migrants, 24-hour oviposition, removal of adults,
165 34-day development period, census) repeated each subsequent generation. The experiment
166 was terminated after seven generations because nearly all the experimental populations had
167 either gone extinct or their growth rates and population sizes had increased, indicative of
168 evolutionary rescue (see Figure 2).

169 In summary, there were 25 groups, each consisting of one source and three
170 experimental populations (one of each treatment). Thus, there were 25 replicates of each
171 treatment, which we split across three temporal blocks (eight groups in blocks 1 and 2, nine in
172 block 3; Figure 1a). Within each group, treatments were set up sequentially, and propagated for
173 the first four generations in the following order to minimize opportunities for experimental
174 error: 1-migrant, 5-migrants, and then the 0-migrant populations. As this was not random, we
175 tested if this order mattered in a follow-up experiment and found no significant effect of

treatment order on the growth rate the following generation (Figure S2). During generations 4 through 7, the experimental protocol was well established, and so we randomized the order of set-up.

Source population dispersal arrays

The immigration recommendations we followed (one or five migrants per generation) are based on either natural dispersal or translocation. Thus, in order to approximate natural dispersal and make our results applicable to either case, we used migrants with higher demonstrated dispersal ability to represent individuals that would be more likely to leave the source patch and move to a new patch in the absence of translocation (Edelaar & Bolnick, 2012; Cote *et al.*, 2022). Dispersal arrays were created to select dispersing individuals. On day 34 during generations 1-6, habitat patches housing source populations were connected to 3-patch dispersal arrays connected with 2mm holes, creating a 4-patch dispersal array. Hereafter, we refer to the original source patch as patch 1 and the subsequent patches, in order, as patches 2-4, which contained media that had been previously used to rear beetles for 35 days to keep resource availability consistent throughout the array. Adult beetles were given 24 hours to disperse out of patch 1. On day 35, all adults were censused in each of the four patches of the dispersal array (Figure S3). Individuals that dispersed to patches 2-4 were used as migrants (hereafter called dispersing migrants) with preference given to individuals that dispersed to the furthest patch (*i.e.,* individuals were first chosen from patch 4, then patch 3, then 2).

In order to maintain the population size of the source populations at 150 to start every generation, growth rates needed to be above the replacement rate. However, growth rates

198 were sometimes lower than expected, which likely occurred due to effects of demographic
199 stochasticity, which strongly influences the *T. castaneum* system (Melbourne & Hastings, 2008).
200 When this happened, populations were supplemented with individuals from the stock
201 population at the start of the oviposition period, after the dispersal period and immigration to
202 the experimental populations occurred. After the first generation, two source populations were
203 initialized with 110 individuals due to limited availability of stock individuals.
204
205 *Common environment and reciprocal transplant experiment*
206 After seven generations, we terminated the rescue experiment, and all extant
207 populations (58 experimental and 25 source populations) were placed at a constant density of
208 40 individuals on standard media for a single generation (hereafter, "common environment"
209 generation; Figure 1c). All adults were kept within their experimental populations, *i.e.,* no
210 mixing occurred within or across treatments. In *T. castaneum,* maternal environmental effects
211 can strongly affect growth rate (Hufbauer *et al.,* 2015; Van Allen & Rudolf, 2013), and so this
212 generation where the environment and density were kept the same for all populations served
213 to standardize maternal effects for the subsequent reciprocal transplant experiment. With only
214 a single generation, we do not standardize grand-maternal effects. However, inference from
215 multigenerational studies suggests that grand maternal effects are relatively weak in *T.*
216 *castaneum* populations (Van Allen & Rudolf, 2013; Hufbauer *et al.,* 2015).
217 In the ninth generation, a reciprocal transplant experiment was completed to assess
218 adaptation to the challenging environment and potential costs to adaptation in the source
219 environment (Figure 1d). We divided individuals from each population into three groups and

220 randomly assigned them to one of three environments: standard media (0ppm, hereafter
221 "source environment"), media containing the concentration of deltamethrin present in the
222 challenging environment of the rescue experiment (2.968ppm, hereafter "experimental
223 environment"), and media containing a higher concentration of deltamethrin (5.641ppm,
224 hereafter "severe environment"). The dose-response curve (Figure S1) indicated that the severe
225 environment would reduce the growth rate of naive populations to near zero. Each replicate
226 patch of these three environmental treatments received 40 beetles, which were allowed 24
227 hours for oviposition, and 34 additional days for development prior to census. Replication
228 within population depended on the number of available individuals following the common
229 environment generation (see Figure S4).
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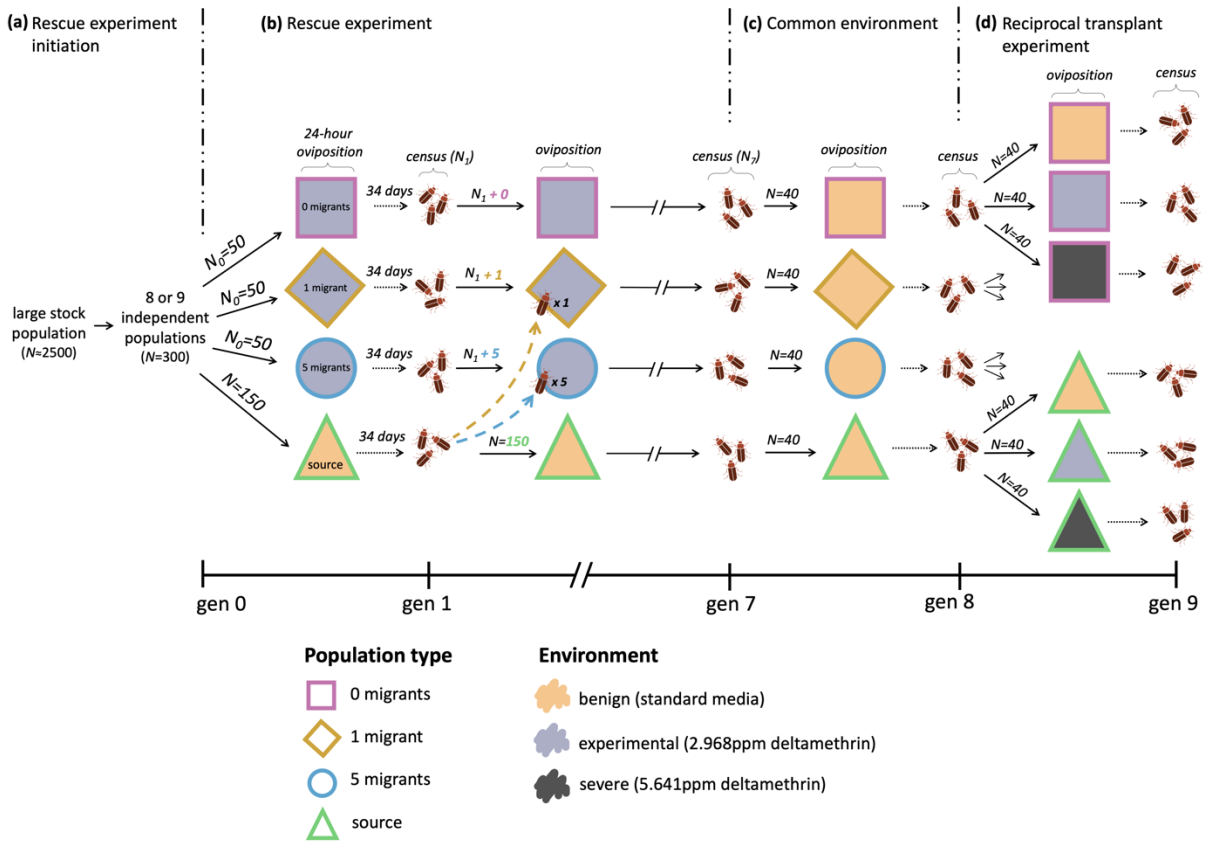


Figure 1. Experimental design for the rescue and reciprocal transplant experiments. a) We began with a large stock population (N≈2500), from which eight or nine independent populations were initiated (N=300). Each of these populations was split into one source population, initiated and then maintained at N=150 individuals, and three experimental populations, initiated with N_0=50 individuals with population size allowed to fluctuate thereafter. The source populations were maintained on a benign environment (tan shading), and the experimental populations were maintained in a challenging environment containing the insecticide deltamethrin (light gray shading). **b)** In the rescue experiment, census events (gens 1 & 7 shown) are indicated with three cartoon beetles, and census occurred every generation on day 35, following a 24-hour oviposition period and 34 days of development

(indicated with dotted arrows). Immigration from the source to the experimental populations (dashed arrows) occurred after each census event at the start of the oviposition period, and three treatments were implemented: 0 migrants (purple square outlines), 1 migrant (yellow diamond outlines), and 5 migrants per generation (blue circle outlines). **c)** All extant populations were placed on a benign, common environment for a single generation following the census in generation seven at a density of *N*=40, with as many replicates created as possible. **d)** In the reciprocal transplant experiment, all populations were split into groups of 40 and placed simultaneously and at constant densities on three different environments: benign, challenging, and severe (dark gray shading). This whole procedure **(a-d)** was repeated across three temporal blocks, with eight replicates in blocks 1 and 2 and nine replicates in block 3.

Data analysis

All statistical analyses were performed in R version 4.1.2 (R Core Team, 2021). Population size at census, which occurred on day 35 during generations 1-7, was used to assess population dynamics throughout the rescue experiment. Populations were considered rescued if they grew to a population size greater than 50, and maintained a growth rate greater than 1 (the replacement rate) for at least two of the final four generations. This allowed for decreases in population size due to negative density dependence. Extinction risk and survival times were quantified using a survival analysis with the *ggsurvfit* function in the *survival* package (Therneau 2021). Extinct populations were defined as those with zero adults at census during the first six generations or one adult by generation seven. Overall extinction rates were calculated as raw percentages with confidence intervals around each percentage for each treatment.

264 Population growth rate was calculated as the mean number of surviving adult offspring
265 per individual in the population, *i.e.,* population size at census each generation (N_t) divided by
266 the number of parents that produced those individuals (parent population size, N_{t-1}), or N_t/N_{t-1}.
267 We evaluated both density-dependent and density-independent growth rates through time
268 using a linear mixed model with the *lme4* package (Bates *et al.*, 2015). We included immigration
269 treatment (0 migrants, 1 migrant, or 5 migrants per generation), time (t) in generations (from 1
270 to 7 generations, categorical), and parent population size (N_{t-1}, integers) as fixed effects, and
271 temporal block (block 1, block 2, block 3), source population (nested within block), and
272 population identity (nested within source population) as random effects. Growth rate data
273 were *log(x+1)* transformed to help satisfy the equal variance assumption. Diagnostic plots were
274 visualized for all models using the *DHARMa* package (Hartig 2022).
275 From this model, we generated estimates of both density-dependent and density-
276 independent population growth rates using the *predict* function with different values for the
277 parent population size (N_{t-1}) each generation. To estimate density-dependent growth rates, we
278 allowed the values for N_{t-1} to vary for each generation and treatment combination, matching
279 the observed mean population sizes at the start of each generation. This estimate was used to
280 describe the population growth rate data and quantify the effects of immigration treatment in
281 a finite habitat by considering the constraints of competition for resources and cannibalism that
282 affected the populations in our experiment (Pointer *et al.*, 2021). To estimate density-
283 independent growth rates from the fitted model, we set N_{t-1} to zero for every generation and
284 treatment, which is equivalent to estimating the y-intercepts when plotting model-estimated
285 growth rate over N_{t-1} (Figure S5). These density-independent estimates allowed us to track

286 adaptation to the environment by revealing the change in intrinsic fitness of individuals in the
287 population unfettered by the effects of density. We expected the density-independent growth
288 rates to be higher than the density-dependent estimates due to the known negative density
289 dependence in *T. castaneum* (Melbourne & Hastings 2008). Confidence intervals for both
290 density-dependent and density-independent growth rate estimates were estimated by
291 parametric bootstrap (percentile method) using the *bootMer* function with 5000 simulations in
292 the *lme4* package (Bates *et al.*, 2015). Comparisons between treatments were made by
293 estimating confidence intervals by parametric bootstrap (percentile method) for the difference
294 in means between treatments within each generation using the *simpleboot* and *boot* packages
295 with 5000 simulations (Peng, 2019; Canty & Ripley, 2022).

296 The timing of evolutionary rescue was assessed in each treatment group using a
297 Generalized Additive Mixed (GAM) model (Wood, 2017) similar to Olazcuaga *et al.* (2023). Only
298 rescued populations were considered in this analysis. We modeled the population size of
299 rescued populations using a negative binomial distribution to account for overdispersion using
300 the package *mgcv* (Wood, 2004, 2011). To allow for the relationship between the population
301 size and time to be nonlinear, we modeled population size as a smooth function of generation
302 with the number of knots set to 4, which was chosen to maximize the adjusted R-squared value.
303 Explanatory variables also included immigration treatment and the interaction of treatment
304 and generation as fixed effects, allowing for a smooth function for each treatment. The full
305 model included population identity and temporal blocks as random effects. The best-fit model
306 was chosen by comparing to reduced models using the *compareML* function from the *itsadug*
307 package (van Rij *et al.*, 2022).

We used data from the reciprocal transplant experiment to further evaluate adaptation to the experimental environment, as well as potential fitness costs of adaptation in a benign environment. Growth rate in the reciprocal transplant experiment was square-root transformed and used as the response variable in a linear mixed effects model that included population type (source, 0-migrant, 1-migrant and 5-migrant), test environment (source, experimental, severe), and the interaction between population type and test environment as fixed effects. Population identity was included as a random effect nested within temporal block. Comparisons within each environment and among immigration treatments were made using 95% bootstrapped confidence intervals (percentile method) for the difference in means, which were generated from 5000 iterations.

3 | Results

Population size and extinction

During the rescue experiment, a majority of populations experienced evolutionary rescue (Figure 2, black lines), and extinction probability was similar for all three treatments (Figure 2, red lines). The difference in the extinction risk was statistically similar across treatments throughout the experiment (Figure S6). Additionally, there was no clear difference in survival time among treatments ($\chi_2^2 = 0.1$, p = 1.00).

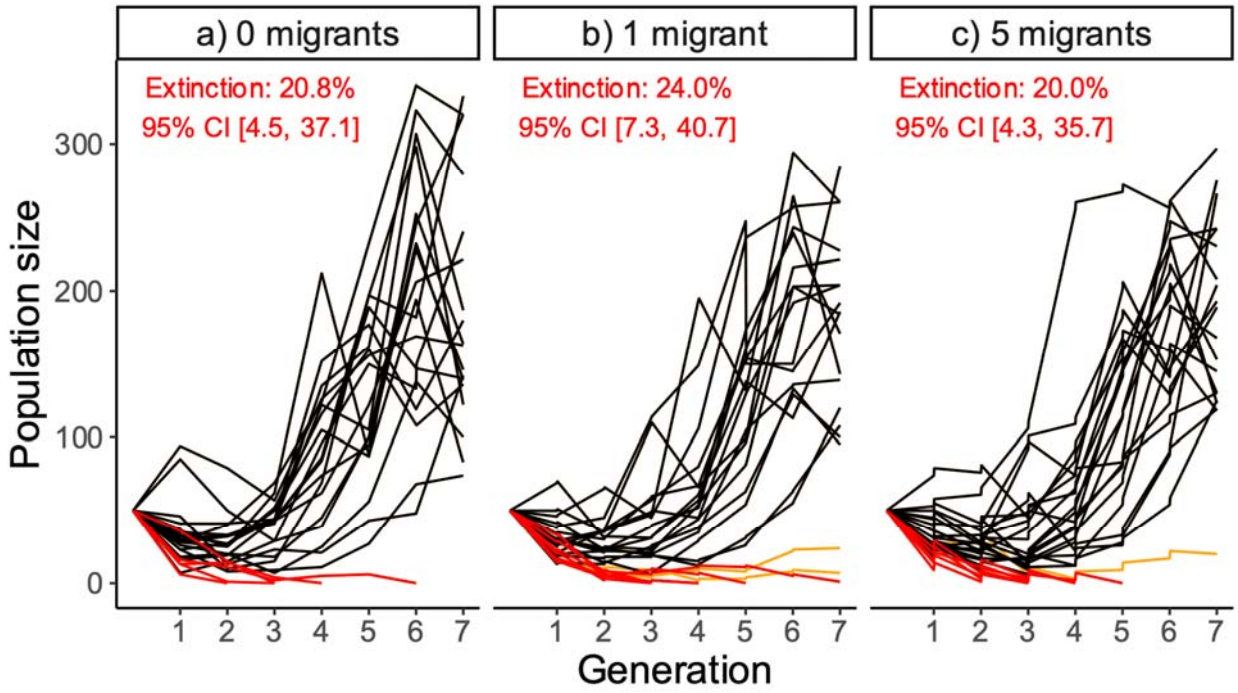
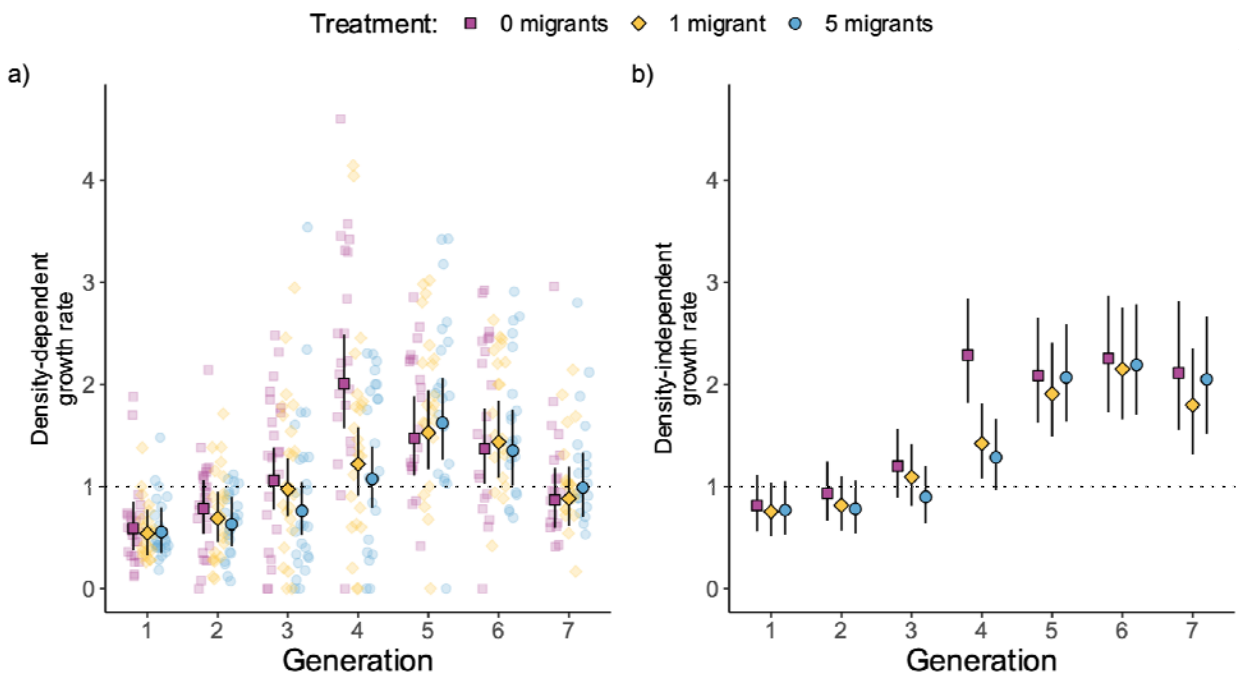


Figure 2. Population size through time in discrete generations for the populations receiving 0, 1, or 5 migrants per generation during the rescue experiment. Population sizes represent the number of adult beetles present at census (day 35 each generation). Each black line represents a rescued population, each orange line represents an extant population that was not considered rescued, and each red line represents a population that went extinct or had a population size of one individual in generation seven. Immigration events are indicated with instantaneous increases in population size, which occurred at the start of the first six generations. The percent of populations that went extinct and simple confidence intervals around each percent are provided.

Growth rate through time

Density-dependent growth rates increased through generation four or five, then decreased again as population density increased (Figure 3a, Table S1). Density-independent growth rates were below or close to the replacement rate (growth rate of 1) in generations 1-3 (Figure 3b; confidence intervals overlap with dashed line). This changed in generation four, when the 0-migrant treatment had a mean density-independent growth rate much larger than the replacement rate, illustrating rapid adaptation within those populations (Figure 3b). In generation four, density-independent growth rate in the 0-migrant populations was on average 0.808 (95% CI [0.207, 1.453]) higher than the 1-migrant populations and 0.969 [0.478, 1.573] higher than the 5-migrant populations. By generation five, all treatments gained and then maintained a mean density-independent growth rate greater than 1, demonstrating adaptation to the challenging environment. The comparison between the density-independent and density-dependent estimates of growth rates suggest that the decrease in the latter by generation seven was likely due to the effects of negative density dependence, which increased as populations grew.



356

357 **Figure 3. Density-dependent growth rate (a) and density-independent growth rate (b) throughout the**

358 **rescue experiment.** Each point outlined in black is the predicted mean for each treatment (purple

359 square = 0 migrants, yellow diamond = 1 migrant per generation, and blue circle = 5 migrants per

360 generation). Error bars are bootstrapped 95% confidence intervals. Points without black outlines are raw

361 growth rate values, and each point represents one population. The dotted line represents a growth rate

362 of 1, the replacement rate. Two estimates for growth rate are presented: a) the density-dependent

363 estimates are predictions from the linear mixed model for each generation and treatment combination,

364 used to incorporate the effects of negative density-dependence and provide estimates that describe the

365 raw data (shown), and b) the density-independent estimates (*i.e.,* predictions using $N_{t-1} = 0$), showing

366 adaptation over time.

367

368 *Timing of evolutionary rescue*

369 Among the rescued populations only (black lines in Figure 2), the analysis of population

370 size with a GAM model revealed a shallow U-shaped curve (Figure 4). The estimated curves

371 from the best-fit model, which included treatment and the interaction of treatment and
372 generation as fixed effects and population identity as a random effect, are shown in Figure 4.
373 The interaction between immigration treatment and generation was significant (, $p <$
374 0.0001), with the 0-migrant populations growing slightly faster than populations receiving
375 migrants (Figure 4). The 1-migrant and 5-migrant treatments maintained similar population
376 trajectories throughout the experiment.
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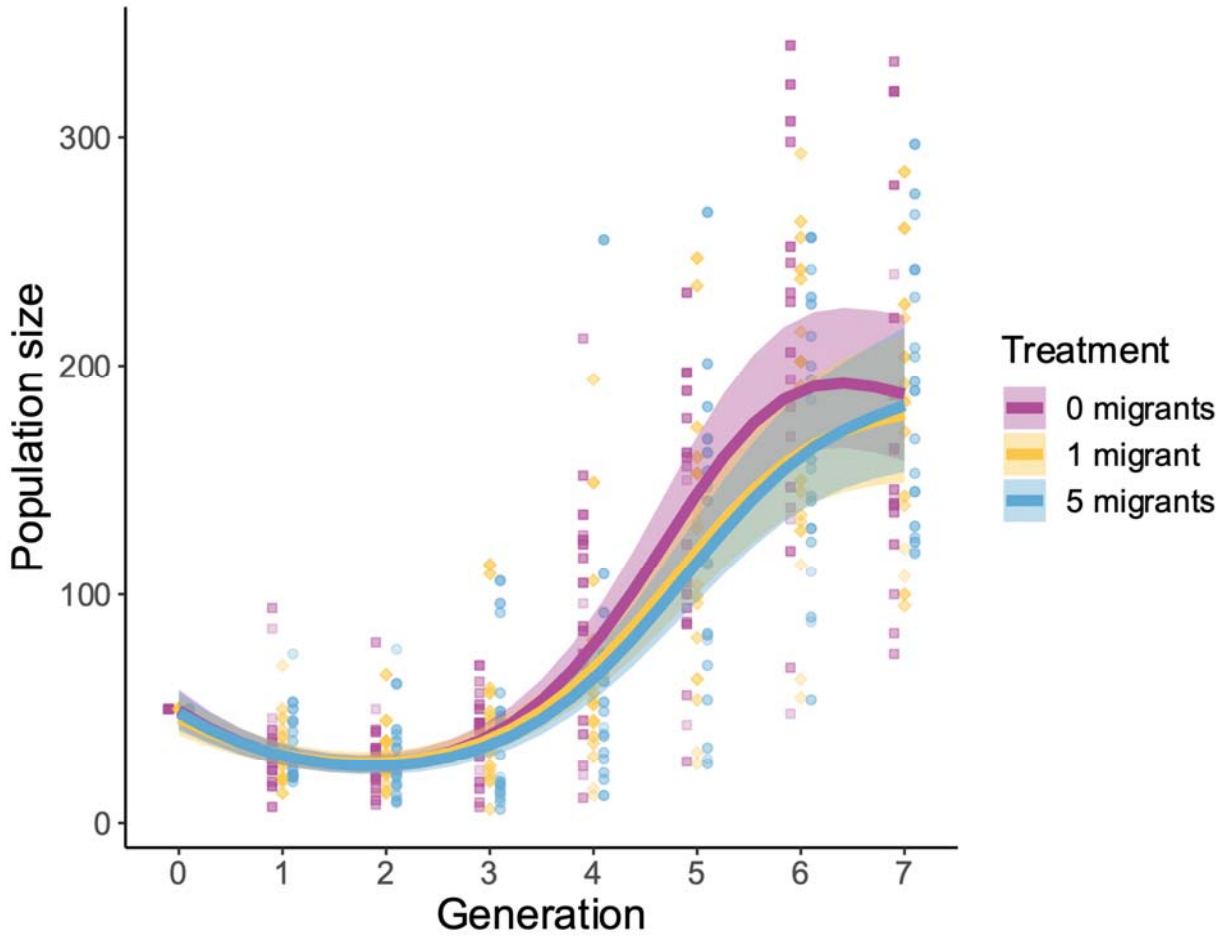

378

379 **Figure 4. Evolutionary rescue analysis.** Population size over time (in generations) of rescued
380 populations receiving 0 migrants (purple line, squares), 1 migrant (yellow line, diamonds), or 5

migrants (blue line, circles) per generation estimated by the best-fit GAM model. Each point represents one population, which are staggered to reduce overlap. The lines are estimates of mean population size for the three immigration treatments, and the shaded region around each line is the 95% confidence interval given by the GAM model.

Adaptation and associated costs

Growth rate in the reciprocal transplant experiment was influenced by an interaction between environment and population type ($F_{6, 168.04} = 25.16$, $p < 0.0001$), in a pattern showing that adaptation to the pesticide was linked to reduced fitness in the benign source environment (Figure 5). In the benign environment, the experimental populations had lower growth rates on average compared to the source populations (confidence intervals are negative and do not overlap with zero: 95% $CI_{0\text{-migrant} - \text{source}}$ [-0.855, -1.628]; 95% $CI_{1\text{-migrant} - \text{source}}$ [-0.911, -1.748]; 95% $CI_{5\text{-migrant} - \text{source}}$ [-0.641, -1.482]), and a higher growth rate in the severe habitat (confidence intervals are positive and do not overlap with zero: 95% $CI_{0\text{-migrant} - \text{source}}$ [0.442, 0.171]; 95% $CI_{1\text{-migrant} - \text{source}}$ [0.424, 0.210]; 95% $CI_{5\text{-migrant} - \text{source}}$ [0.464, 0.216]). Additionally, the 5-migrant populations performed marginally better than the 0-migrant populations in the experimental environment (95% $CI_{0\text{-migrant} - 5\text{-migrant}}$ [-0.0675, 0.572]).

Unexpectedly, the experimental and source populations performed similarly in the challenging experimental environment (Figure 5, all confidence intervals of the differences overlap with zero: 95% $CI_{0\text{-migrant} - \text{source}}$ [-0.404, 0.209]; 95% $CI_{1\text{-migrant} - \text{source}}$ [-0.220, 0.403]; 95% $CI_{5\text{-migrant} - \text{source}}$ [-0.153, 0.452]). The experimental populations did not perform as well as

403 expected based on their growth rates at the end of the rescue experiment (Figure 3). Our
404 expectation for growth rates were based on predictions from the linear mixed model created
405 for the rescue experiment with N_{t-1} = 40 (the number of parents used in the reciprocal
406 transplant) and gen = 7 (the time closest to the start of the reciprocal transplant). Those
407 predicted values were 1.80, 95% CI [1.32, 2.39], 1.52 [1.12, 2.00], and 1.75 [1.31, 2.26] for the
408 0-migrant, 1-migrant, and 5-migrant treatments, respectively. The average growth rates we
409 observed in the reciprocal transplant experiment in the experimental environment were on
410 average 40% lower than these estimates, with the means 0.85 [0.64, 1.09], 1.04 [0.80, 1.32],
411 1.10 [0.86, 1.35].

412 Furthermore, source populations did not perform as poorly as expected in the
413 experimental environment. We expected them to perform similarly to the first generation of
414 the experimental populations in the rescue experiment prior to adaptation. The prediction
415 given N_{t-1} = 40 was 0.63 [0.38, 0.83] for the 0-migrant populations at the start of the rescue
416 experiment, but the mean growth rate of source populations was about 46% higher at 0.92
417 [0.72, 1.15].

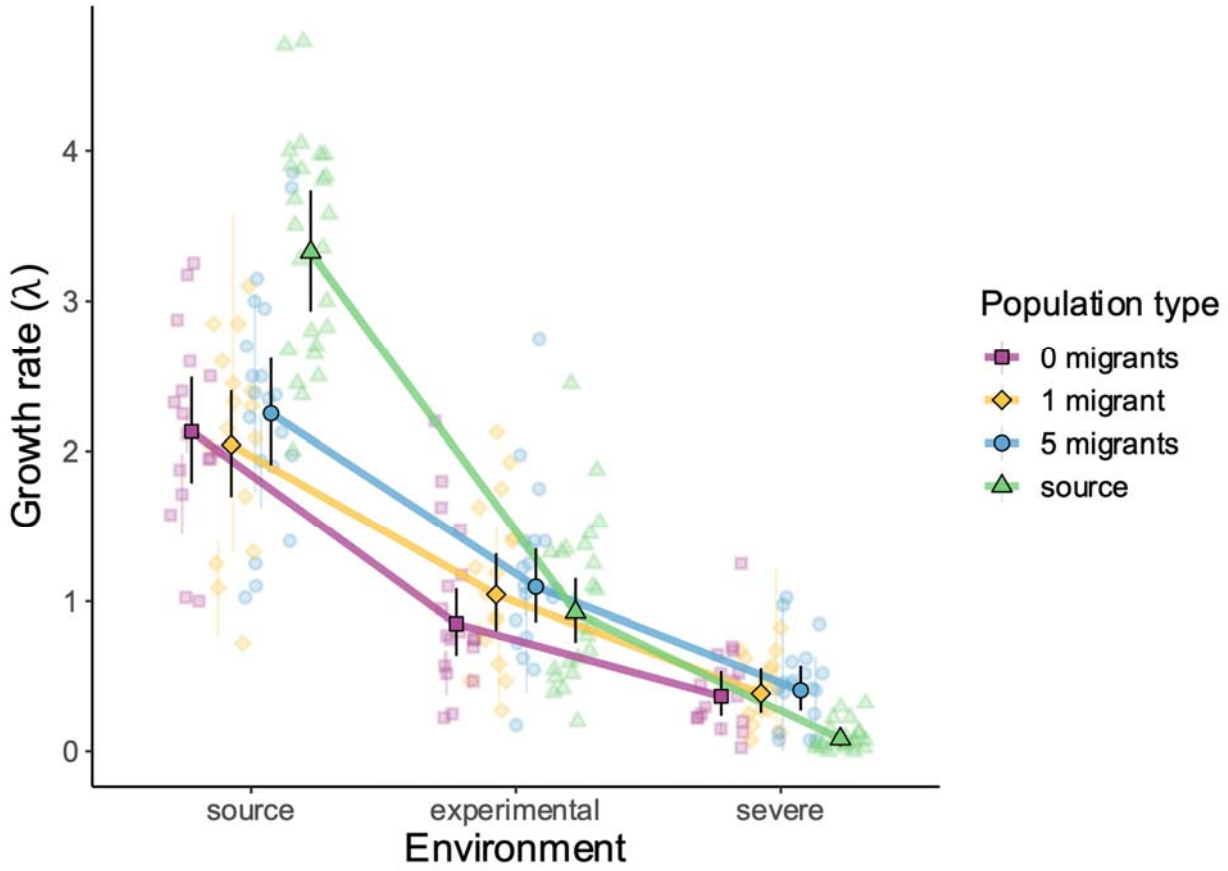

Figure 5. Reciprocal transplant experiment results. Growth rate was measured in three environments: the source environment (standard dry media), the challenging experimental environment, and a severe environment containing a higher concentration of insecticide. Each point outlined in black is the model-estimated mean for each population type (purple squares = 0-migrant populations, yellow diamonds = 1-migrant populations, blue circles = 5-migrant populations, and green triangles = source populations). Black error bars are bootstrapped 95% confidence intervals; for the source populations in the severe environment (green triangles), the black error bars are not visible because they are too small to be seen. Thick lines connecting these points emphasize the treatment-environment interaction. Each point without the black outline represents a single population, with the light error bars representing raw 95%

429 confidence intervals across replicates; points without error bars have no replicates. Points are
430 staggered and jittered to reduce overlap.
431
432

433 4 | Discussion

434 Our study evaluated the effects of different immigration rates on adaptation to a new,
435 challenging environment using experimental populations of *Tribolium castaneum*. We
436 evaluated differences between initially declining populations receiving two immigration rates
437 (one or five migrants per generation) with isolated populations that received no immigration.
438 The migrants were not adapted to the challenging environment, allowing us to examine the
439 degree to which immigration can limit adaptation (Savolainen *et al.*, 2013). While the
440 experimental populations declined in size initially, leading some populations to go extinct,
441 around 80% of populations were able to persist for the duration of the seven-generation
442 experiment. Most of these populations had growth rates above the replacement rate and had
443 large population sizes, characteristic of evolutionary rescue. We also found that extinction rate
444 was similar regardless of immigration. Notably, growth rates increased one generation sooner
445 in the isolated populations compared to those with immigration. This delay was brief, as the
446 following generation, growth rates were comparable across the different immigration
447 treatments. We also tested for adaptation using a reciprocal transplant experiment. While
448 some of the results from that experiment were unexpected, overall it provided evidence that
449 our experimental populations were adapted to the insecticide that created the challenging

environment. There was also a cost to that adaptation, evident from the experimental populations having lower growth rates than the sources in the benign environment.

Observed delay in the increase in growth rate: possible mechanisms

Populations without immigration gained a much higher density-independent growth rate (more than doubling on average) compared to the populations with immigration (near or just above the replacement rate) in the fourth experimental generation. The following generation, all populations grew at a similar rate regardless of immigration. The delay we observed may have been due to *i)* differences in density dependence, *ii)* phenotypic plasticity, or *iii)* outcrossing with naive migrants slowing adaptation to the challenging environment.

First, theory shows that reduced negative density dependence in a degraded habitat may enhance rescue (Uecker *et al.*, 2014; Czuppon *et al.*, 2021). In our system, population densities were low in the experimental populations in the initial generations, and thus negative density-dependent processes were likely relatively weak. The added immigrants increased density slightly relative to the treatment without migrants, and this could have contributed to lower growth rates. However, if this were the case, the populations receiving five migrants each generation should have experienced further reductions in growth than the populations receiving one per generation, as their density was increasing by a greater amount (31% increase for the 5-migrant populations on average compared to a 7% increase for the 1-migrant populations). Thus, density dependence is an unlikely explanation for the one-generation delay in increased growth rates.

471 Second, growth rate in the challenging environment could be influenced by phenotypic
472 plasticity, which could have led to the lag in increased growth rates. Recently, it has been
473 shown that plasticity in insects can be mediated by associated microbes (Kolodny &
474 Schulenburg, 2020). Variation in the microbial communities in host insects is high (Lange *et al.,*
475 2023), and the microbial community can evolve, which can facilitate persistence in new and
476 challenging environments (Kolodny & Schulenburg, 2020). Microbes, therefore, can serve as an
477 important source of adaptive phenotypic plasticity (Kolodny & Schulenburg, 2020; Ghalambor
478 *et al.,* 2007). Insect-associated microbes can be transmitted from parent to offspring (Lange *et*
479 *al.,* 2023), indicating that plasticity could be multi-generational. If the microbial community
480 influences fitness in the presence of deltamethrin, it is possible that plasticity and adaptation in
481 the microbiome helped facilitate the observed increase in growth rate during the rescue
482 experiment. The delay in increase for populations with immigration could be due to the
483 microbiome from the benign environment being continually re-introduced by the migrants.
484 Third, migration is well known as a constraint to adaptation (Slatkin, 1987). For example,
485 theory has shown that immigration between different habitat types can limit local adaptation
486 through genetic swamping of adaptive alleles (Lenormand, 2002; Bolnick & Nosil, 2007), and
487 reduce the probability (Schiffers *et al.*, 2013) or speed (Holt & Barfield, 2015) of evolutionary
488 rescue because of maladaptation in the new environment. It seems likely that migration of
489 naive individuals slowed adaptation to the novel environment, which may have been enhanced
490 by the mating behavior of *T. castaneum*. In this species, males reared in high-quality
491 environments have increased mating rate and insemination success compared to males reared
492 in poor environments (Lewis *et al.*, 2012). Thus, male migrants from the benign source

493 environment may have successfully mated with multiple females in the experimental patches,
494 enhancing the rate of gene flow of maladapted alleles. Female migrants would have also likely
495 spread maladapted alleles, as a single female *T. castaneum* can lay over 10 eggs per day (Pray
496 et al., 1949). Thus, regardless of sex, outcrossing with the naive migrants likely contributed to
497 the brief delay in growth rate increase. Given the strength of selection pressure, however,
498 adaptation likely enabled growth rates to increase in the following generation, which we
499 observed on average across all treatments.

500 We suggest that the increases in population size and growth rate, likely through
501 adaptation and possibly aided by adaptive plasticity, can be described as evolutionary rescue.
502 We further evaluate the effects of adaptation *vs.* plasticity when we discuss the reciprocal
503 transplant experiment below. The finding that rescue can occur with intermediate levels of
504 immigration of not adapted individuals is supported by several modeling studies that assess
505 evolutionary rescue in heterogenous, structured systems (Gomulkiewicz *et al.*, 1999; Uecker *et*
506 *al.*, 2014; Tomasini & Peischl, 2020; Czuppon *et al.*, 2021). This result has also been shown in a
507 case study of Trinidadian guppies, where rescue was able to occur with gene flow from a
508 maladapted source population (Fitzpatrick *et al.,* 2014, 2020).

509

510 *Observed delay in rescue: implications*

511 Our finding of only a brief (one generation) delay of adaptation has practical
512 implications. Such delayed adaptation could be detrimental to populations at imminent risk of
513 extinction due to small size (Gomulkiewicz & Holt, 1995; Melbourne & Hastings, 2008).
514 However, for populations not at immediate risk of extinction, such a delay may be

515 inconsequential, and worth the potential increase in genetic diversity that occurs through
516 immigration. Such increased diversity may prevent inbreeding depression from developing, and
517 improve the ability of the population to adapt to future environmental changes (Frankham *et*
518 *al.,* 2019).

519 Thus, these results suggest that naive migrants can be suitable to use in management
520 efforts. Furthermore, they are often more feasible to obtain, either from captive rearing
521 programs (Crone *et al.,* 2007) or from large source populations living in a different habitat (*e.g.,*
522 different host plants in Bisschop *et al.,* 2019) or geographic region (*e.g.,* isolated guppy
523 populations in Fitzpatrick *et al.,* 2020), when habitat matching is not possible.

524 Additionally, our findings are consistent with refuge strategies to delay evolution of pest
525 resistance to toxins, such as in crops genetically modified to express *Bacillus thuringiensis* (*Bt*).
526 The immigration of susceptible individuals from sections of fields planted in non-*Bt* crops, or
527 growing crops in areas with plentiful alternative host plants for the pest has proven to be highly
528 effective (Carrière *et al.*, 2012). In those situations, particularly with 50% or more of a pest
529 population being susceptible, resistance can be successfully managed for multiple generations
530 (Tabashnik *et al.*, 2008). Given our much smaller proportion of susceptible individuals, it makes
531 sense that the delay of the evolution of resistance was brief.

532

533 *Reciprocal transplant experiment: interpretation of unexpected results*

534 The two most straightforward takeaways from the reciprocal transplant experiment are
535 *i)* the experimental populations had higher growth rates than the source populations in the
536 severe environment, suggesting they had adapted to the insecticide used to create the

537 challenging environment in the rescue experiment and *ii)* the experimental populations had
538 lower growth rates in the benign environment than the source populations, suggesting that
539 there was a cost associated with adaptation to the insecticide. However, the source populations
540 had higher than expected growth rates in the experimental environment, and the experimental
541 populations had lower than expected growth rates in the experimental environment
542 inconsistent with the hypothesis of adaptation. We next discuss *i)* how experimental challenges
543 might have affected the source populations, *ii)* how the common environment generation could
544 have reduced growth rates by eliminating effects of plasticity, and *iii)* how multiple pathways of
545 adaptation in the experimental populations could explain their performance in the reciprocal
546 transplant experiment.

547 First, the high performance of the source populations in the experimental environment
548 could be due to challenges in implementing the experimental design. For instance, accidental
549 contamination of the source population with deltamethrin, or accidental gene flow from the
550 experimental to the source populations could have occurred during the rescue experiment. We
551 took great care to avoid both forms of contamination, but if contamination nevertheless
552 occurred, it could have allowed the source populations to adapt to low concentrations of the
553 insecticide. The source populations were not adapted to high concentrations of the pesticide,
554 as evidenced by their performance in the severe environment.

555 A second consideration is that the common environment generation could have masked
556 differences in growth rates, if they were initially caused by plasticity. As described above,
557 microbiome-mediated phenotypic plasticity could have contributed to the increase in growth
558 rate in the rescue experiment (Lange *et al.,* 2023). If microbiome-mediated plasticity drove

559 performance, the generation in a common environment prior to the reciprocal transplant
560 experiment would have alleviated selection pressure and reduced the ability of the
561 experimental populations to tolerate the insecticide. This could help explain the similar
562 performance of the experimental and source populations in the experimental environment.
563 However, there does not appear to be strong evidence for the masking of phenotypic plasticity
564 in the severe or benign environments given that the experimental populations had a much
565 higher growth rate than the sources in the severe environment and a lower growth rate in the
566 benign. We suggest that those differences can be explained by our last point, adaptation, which
567 is discussed below.

568 Finally, adaptation to deltamethrin likely explains the relative performance of the
569 populations in the three environments. More specifically, it is possible that the experimental
570 populations adapted through more than one mechanism during the rescue experiment. First,
571 adaptation to detoxify the insecticide compound likely occurred, as rapid evolutionary
572 responses to deltamethrin is well documented in pest populations of *T. castaneum* (Collins,
573 1998; Stuart *et al.*, 1998) through the increased expression of cytochrome P450 enzymes (Kalsi
574 & Palli, 2015). In other insect species, this mechanism of resistance to deltamethrin has a
575 confirmed cost (Tchouakui *et al.*, 2020), and such a cost is consistent with the reduced
576 performance of experimental populations in the benign environment. The second way that
577 populations could have adapted is by increasing the rate of egg cannibalism to supplement
578 their diets, as cannibalism propensity is a genetically based trait that can evolve (Stevens, 1989)
579 that has been shown to enhance fitness in challenging environments (Via, 1999; Pointer *et al.*,
580 2021). These potential pathways, evolution of increased detoxification and increased

581 cannibalism, are not mutually exclusive, and both may have facilitated increased population
582 growth in the experimental environment during the rescue experiment. Then, in the reciprocal
583 transplant experiment, the populations were initiated at lower density (40 parents) in the three
584 environments. In this lower density environment, the number of eggs available for cannibalizing
585 was reduced compared to what was available in the latter half of the rescue experiment (in
586 generations 4-7, N_{t-1} > 150 on average). Thus, the decreased density reduced the eggs available
587 for cannibalism, limiting one of the adaptive pathways and reducing overall growth rates of the
588 experimental populations in the experimental environment. In the severe environment, growth
589 rates of the experimental populations were low but still greater than the source populations,
590 which can be explained primarily by their evolved ability to detoxify the pesticide.
591
592 *Considerations for future experiments*
593 The bottleneck events that occurred during the rescue experiment, some as small as 10
594 individuals, would likely have longer-term consequences (Frankham *et al.*, 1999; Olazcuaga *et*
595 *al.*, 2023). Census population size in a wide range of natural populations is almost always larger
596 than the effective population size (the median N_e/N_c ratio across plant and animal taxa is
597 approximately 0.1; for *T. castaneum,* it is estimated to be between 0.5-0.9) and bottlenecks of
598 N_e < 50 can result in reductions in genetic variation and increases in inbreeding (Wade, 1980;
599 Jamieson & Allendorf, 2012). For this reason, we suggest that future work examine the effects
600 of demographic bottlenecks of this severity by propagating experiments like this for longer (*i.e.,*
601 more than 8 generations). Longer-term experiments would be able to test the hypothesis that
602 the benefits of immigration become more apparent over longer time scales. Such experiments

603 would help shed light on the longer-term impacts of immigration for small and isolated
604 populations, an important question when considering whether to implement translocations in
605 declining natural populations (Frankham, 2015, 2016). Genomic methods would also provide
606 important insights for monitoring the genetic contributions of migrants, the genetic
607 differentiation between the experimental and source populations, and for confirming the genes
608 contributing to adaptation (Whiteley *et al.*, 2015). Genomics would also allow us to more
609 clearly differentiate between adaptation and plasticity (Koch & Guillaume, 2020).
610
611 *Conclusions*
612 This study explored the effects of immigration of naive individuals on large, outbred,
613 and recently diverged populations following population decline due to sudden environmental
614 degradation. To our knowledge, we are the first to experimentally test the effects of the
615 recommended immigration rates of one compared to five migrants per generation on
616 adaptation in a challenging environment. We found that though immigration slowed adaptation
617 in the short-term, extant populations with and without immigration were on average equally
618 well adapted to the challenging conditions after seven generations. Thus, we confirm the
619 findings of previous theoretical work (Tomasini & Peischl, 2020; Czuppon *et al.*, 2021) by
620 demonstrating experimentally that evolutionary rescue can occur both with and without
621 immigration following severe environmental change.
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624

Data Availability Statement

Data and R scripts are publicly available on the Dryad Digital Repository (https://doi.org/10.5061/dryad.79cnp5j3c).

Conflict of Interest

The authors declare no conflicts of interest.

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